

CLAIMS

1. Polynucleotide sequence encoding a geminivirus-derived aminoacid sequence, said polynucleotide sequence being characterised in that is not or is an ineffective target of the viral post-transcriptional silencing and has:

a) an homology at nucleotidic level below or equal at 90% with respect to the corresponding gene sequence of the geminiviruses against which the resistance is required;

b) a continuous homology in the transcribed RNA, with respect to the corresponding gene sequence of the geminiviruses, below or equal to 17 nucleotides;

c) a maximum length of the sequence containing a single substitution with respect to the corresponding gene sequence of the geminiviruses not higher than 30 nucleotides

said polynucleotide sequence being able to confer to the plants, tissues or plant cells therewith transformed, a lasting resistance against the geminiviruses.

2. Sequence according to claim 1, wherein the homology at the nucleotidic level with respect to the corresponding gene sequence of the geminivirus is below or equal to 80%.

3. Sequence according to claim 1, wherein the homology at the nucleotidic level with respect to the corresponding gene sequence of the geminivirus is below or equal to 70%.

4. Sequence according to each of the preceding claims, wherein the continuous homology in the transcribed RNA with respect to the gene sequence of the geminiviruses is below or equal to 8 nucleotides.

5. Sequence according to each of the preceding claims, wherein the continuous homology in the transcribed RNA with respect to the gene sequence of the geminiviruses is below or equal to 5 nucleotides.

6. Sequence according to each of the preceding claims, wherein the maximum length of the sequence containing a single substitution with respect to the corresponding gene sequence of the geminiviruses is not more than 20 nucleotides.

7. Sequence according to each of the preceding claims, wherein the maximum length of the sequence containing a single substitution with respect to the corresponding gene sequence of the geminiviruses is not more than 9 nucleotides.

8. Sequence according to each of the preceding claims, wherein the polynucleotide sequence has been mutated or it is a wild-type sequence selected from geminivirus so as to differ at the nucleotidic level with respect to the corresponding genomic sequence of the geminivirus against which a resistance is required according to each of the preceding claims.

9. Sequence according to each of the preceding claims, wherein the geminiviruses are selected from the group consisting of species of *Mastrevirus*, *Curtovirus*, *Begomovirus* and *Topocuvirus* and isolates thereof.

10. Sequence according to claim 9, wherein *Begomoviruses* species are selected from the group consisting of TYLCCNV, TYLCGV, TYLCMaIV, TYLCSV, TYLCTHV, TYLCV, ACMV, BGMV, CaLCuV, ToCMoV, TGMV, ToGMoV, ToMHV, ToMoTV, ToMoV, ToRMV, ToSLCV, ToSRV, Cotton leaf curl (CLCrV, CLCuAV, ClCuGV, CLCuKV, CLCuMV, CLCuRV), East African cassava mosaic (EACMCV, EACMMV, EACMV, EACMZV), Potato yellow mosaic (PYMPV, PYMTV, PYMV), Squash leaf curl (SLCCNV, SLCV, SLCYV), Sweet potato leaf curl (SPLCGV, SPLCV), Tobacco leaf curl (TbLCJV, TbLCKoV, TbLCYNV, TbLCZV), Tomato leaf curl (ToLCBV, ToLCBDV, ToLCGV, ToLCKV, ToLCLV, ToLCMV, ToLCNDV, ToLCSLV, ToLCTWV, ToLCVV, ToLCV) and isolates thereof.

11. Sequence according to claim 9, wherein the species belonging to the genus *Mastrevirus*, *Curtovirus*, *Topocuvirus* are selected from the group consisting of WDV, MSV, SSV, BYDV, TYDV, BCTV and isolates thereof.

12. Sequence according to claim 1, wherein the gene sequence is selected from the group consisting of C1/AL1/AC1, C2/AL2/AC2, C3/AL3/AC3, C4/AL4/AC4, V1/AR1/AV1, V2/AR2/AV2, BC1/BL1 and BV1/BR1, belonging to the geminiviruses.

13. Sequence according to claim 12, wherein C1/AL1/AC1 gene sequence is from the geminiviruses, as defined according to claims 10 and 11.

14. Sequence according to claim 1, wherein the geminivirus aminoacidic sequence is a pathogen-derived protein able to confer resistance against the geminiviruses to the plants expressing the protein.

15. Sequence according to claim 14, wherein the protein is selected from the group consisting of capsid protein, replication associated

viral protein (Rep), proteins encoded by the genes C2/AL2/AC2, C3/AL3/AC3, C4/AL4/AC4, V2/AR2/AV2, BC1/BL1 and BV1/BR1.

5 16. Sequence according to claim 1, wherein the plants, tissues or cells thereof belong to the group consisting of tomato, pepper, tobacco, squash, manioc, sweet potato, cotton, melon, potato, soybean, wine, corn, wheat, sugar cane, bean, beet.

10 17. Polynucleotide sequence encoding a geminivirus-derived aminoacid sequence, said polynucleotide sequence being characterised in that is not or is an ineffective target of the viral post-transcriptional silencing and has homology even equal to 100% with respect to the sequence of the geminivirus against which a resistance is required and is suitably shortened to be under represented in the siRNAs population with respect to the original sequence.

15 18. Construct comprising an heterologous polynucleotide sequence containing in the 5'-3' direction:

a) a polynucleotide sequence acting as promoter in said plant or tissue or transformed cells;

b) a non translated polynucleotide sequence positioned 5' of the encoding region;

20 c) a polynucleotide sequence as defined according to claims from 1 to 17, a fragment or a variant thereof;

d) a sequence acting as transcription terminator, positioned 3' with respect to said polynucleotide sequence.

25 19. Expression vector comprising the construct as defined according to claim 18.

20. Transgenic plant, tissue or plant cells thereof, comprising in their genome a polynucleotide sequence as defined according to claims from 1 to 17.

30 21. Progeny of the plants and plant tissues according to the claim 20.

22. Seed comprising in its genome a polynucleotide sequence as defined according to claims from 1 to 17.

35 23. Method for the preparation of transgenic plants, plant tissue or cells thereof long lasting resistant against geminiviruses including the following steps:

a) identification or selection of a viral gene sequence encoding an aminoacid sequence able to confer resistance against geminiviruses;

b) mutagenesis or choice of the viral gene sequence so as to make it an ineffective target of the post-transcriptional silencing induced by infecting geminivirus;

5 c) insertion of the geminivirus gene sequence mutated or chosen in the step b) in the plant, plant tissue or cell thereof using a construct as defined according to claim 18.

24. Method according to claim 23, wherein by mutagenesis the homology at the nucleotidic level with respect to the gene sequence of the geminivirus against which resistance is required is maintained below or equal to 90 %, distributed in such way that the continuous homology in the transcribed RNA with respect to the corresponding gene sequence of the geminiviruses is below or equal to 17 nucleotides and the maximum length of the sequence containing a single substitution with respect to the corresponding gene sequence of the geminiviruses is not higher than 30 nucleotides.

25. Method according to anyone of the claims 23 and 24, wherein by mutagenesis an homology at the nucleotidic level with respect to the gene sequence of geminivirus is maintained below or equal to 80%.

26. Method according to anyone of the claims 23 and 24, wherein by mutagenesis an homology at a nucleotidic level with respect to the gene sequence of geminivirus is maintained below or equal to 70%.

27. Method according to anyone of the claims from 23 to 26, wherein by mutagenesis a continuous homology at the nucleotidic level with respect to the gene sequence of geminivirus is maintained below or equal to 8 nucleotides.

28. Method according to anyone of the claims from 23 to 27, wherein by mutagenesis a continuous homology at the nucleotidic level with respect to the gene sequence of geminivirus is maintained below or equal to 5 nucleotides .

29. Method according to anyone of the claims from 23 to 28, wherein the maximum length of the sequence containing a single substitution with respect to the corresponding gene sequence of geminiviruses is no more than 20 nucleotides.

30. Method according to anyone of the claims from 23 to 29, wherein the maximum length of the sequence containing a single substitution with respect to the corresponding gene sequence of geminiviruses is no more than 9 nucleotides.

31. Method according to anyone of the claims from 23 to 30, wherein the mutagenesis consists of silent point mutations or deletions and/or insertions and/or substitutions.

5 32. Method according to anyone of the claims from 23 to 31, wherein the mutagenesis in step b) consists of deletions of the 5' or 3' regions of the viral gene sequence of step a) until the identification of the minimum region of said gene sequence that is under represented with respect to the sequence encoding the wild-type protein, in the population of the interfering siRNAs and that said truncated protein maintains the
10 ability to confer resistance against geminiviruses.

33. Method according to claim 32, wherein the viral gene sequence of step a) is the C1/AL1/AC1 gene.

34. Method according to claim 32, wherein the C1/AL1/AC1 gene is a TYLCSV gene.

15 35. Method according to claim 32, wherein the aminoacid sequence is a truncated protein with respect to the viral wild-type protein.

36. Method according to anyone of the claims from 32 to 35 in which the viral gene sequence made not target or ineffective target of the post-transcriptional silencing is the SEQ ID No 8.

20 37. Method according to anyone of the claims from 32 to 36, wherein the truncated protein is Rep-130 (SEQ ID No 9).

38. Method according to anyone of the claims from 23 to 31, wherein the mutagenesis in step b) consists of silent point mutations of the viral gene sequence of step a) to maintain the ability of the aminoacid
25 sequence, encoded by the same, to confer resistance against geminiviruses and not to be or to be an ineffective target of the post-transcriptional silencing.

39. Method according to claim 38, wherein the viral gene sequence of step a) is the V1/AR1/AV1 (CP) gene.

30 40. Method according to claim 39, wherein the V1/AR1/AV1 (CP) gene is a TYLCSV gene.

41. Method according to anyone of the claims from 38 to 40, wherein the viral gene sequence made not target or ineffective target of the post-transcriptional silencing is the SEQ ID No 8.

35 42. Method according to claim 38, wherein the viral gene sequence of step a) is C1/AL1/AC1 of TYLCSV.

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43. Method according to claim 42, wherein the viral gene sequence made not target or ineffective target of the post-transcriptional silencing is the SEQ ID No 2 or the SEQ ID No 4.